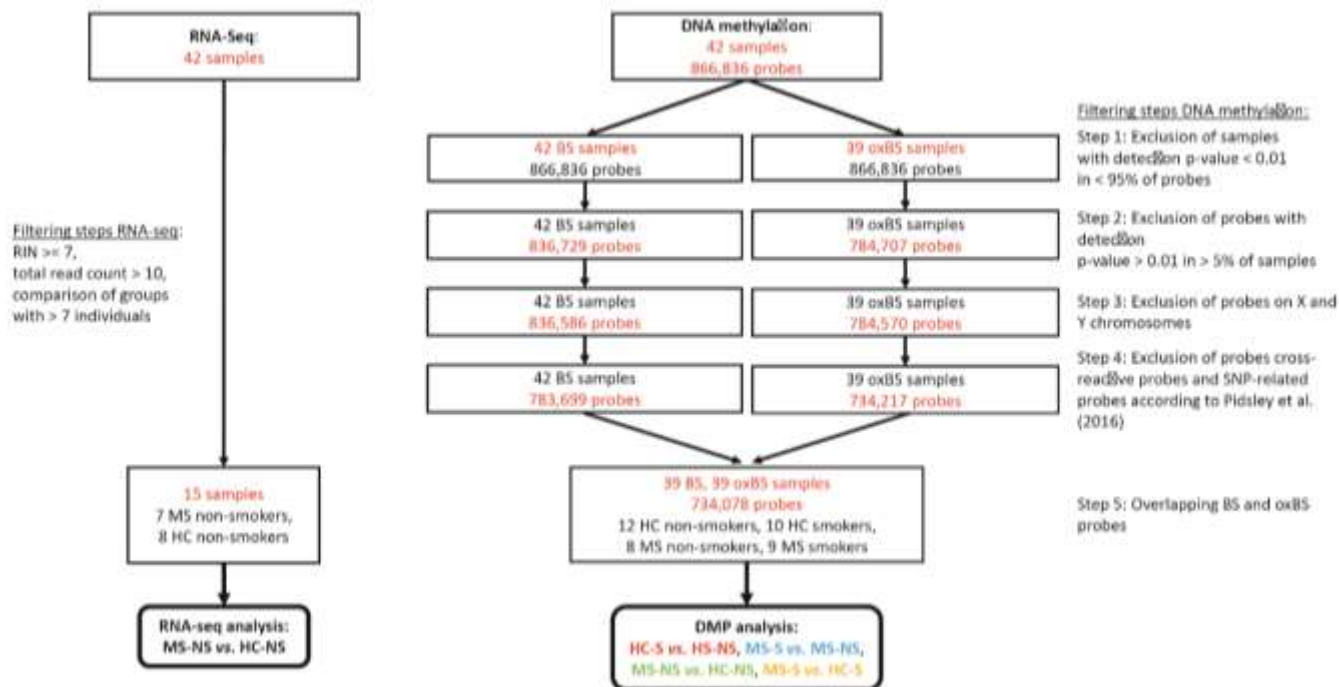


Supplementary Figure 1

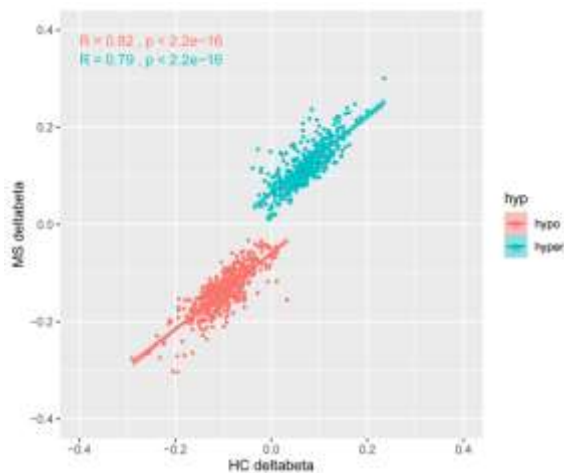
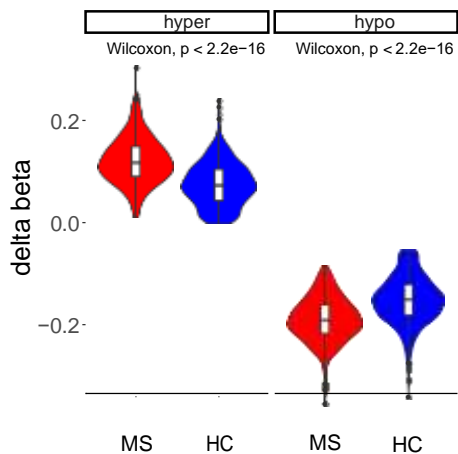


Supplementary Figure 1. Analytical workflow

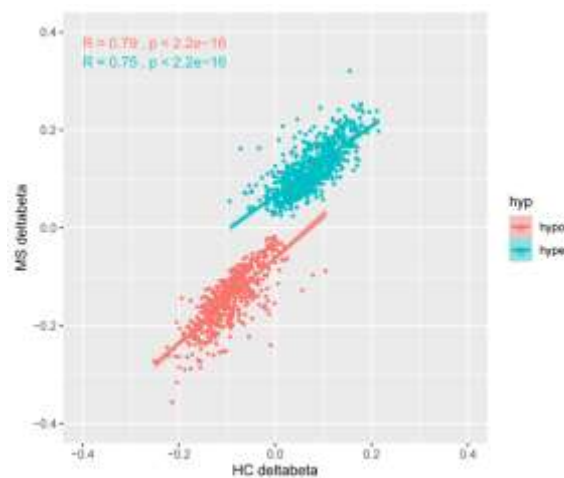
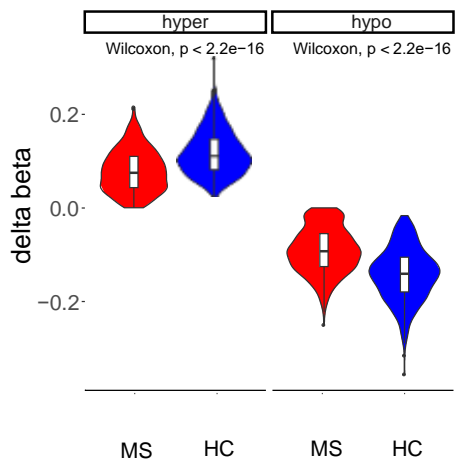
The schematic is representing the filtering steps performed before statistical analyses of RNA-seq and DNA methylation/hydroxymethylation data. Healthy controls (HC) and multiple sclerosis (MS) patients were divided into groups of smokers (S) and non-smokers (NS).

REVISED Supplementary Figure 2

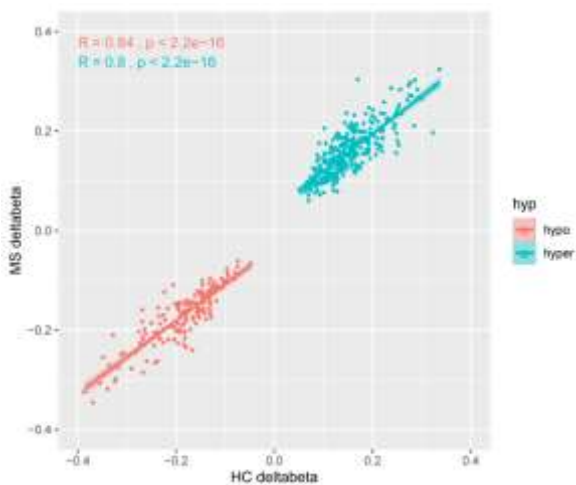
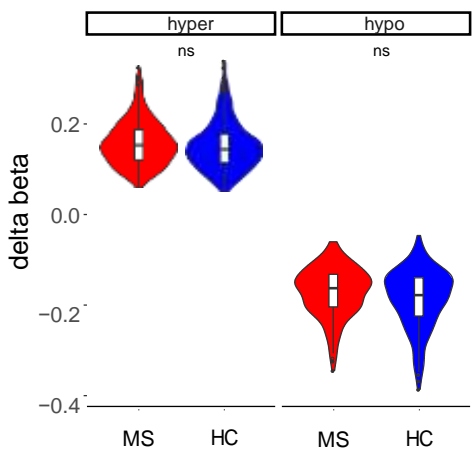
a



b

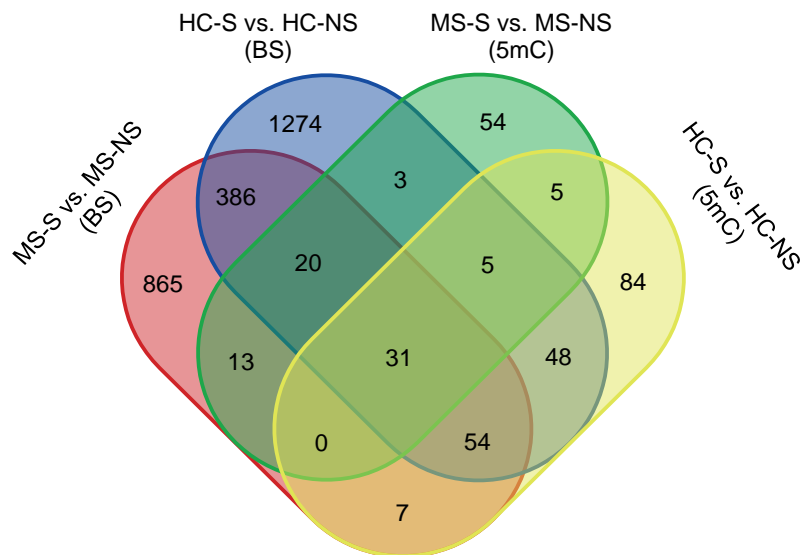


c



Supplementary Figure 2 (end)

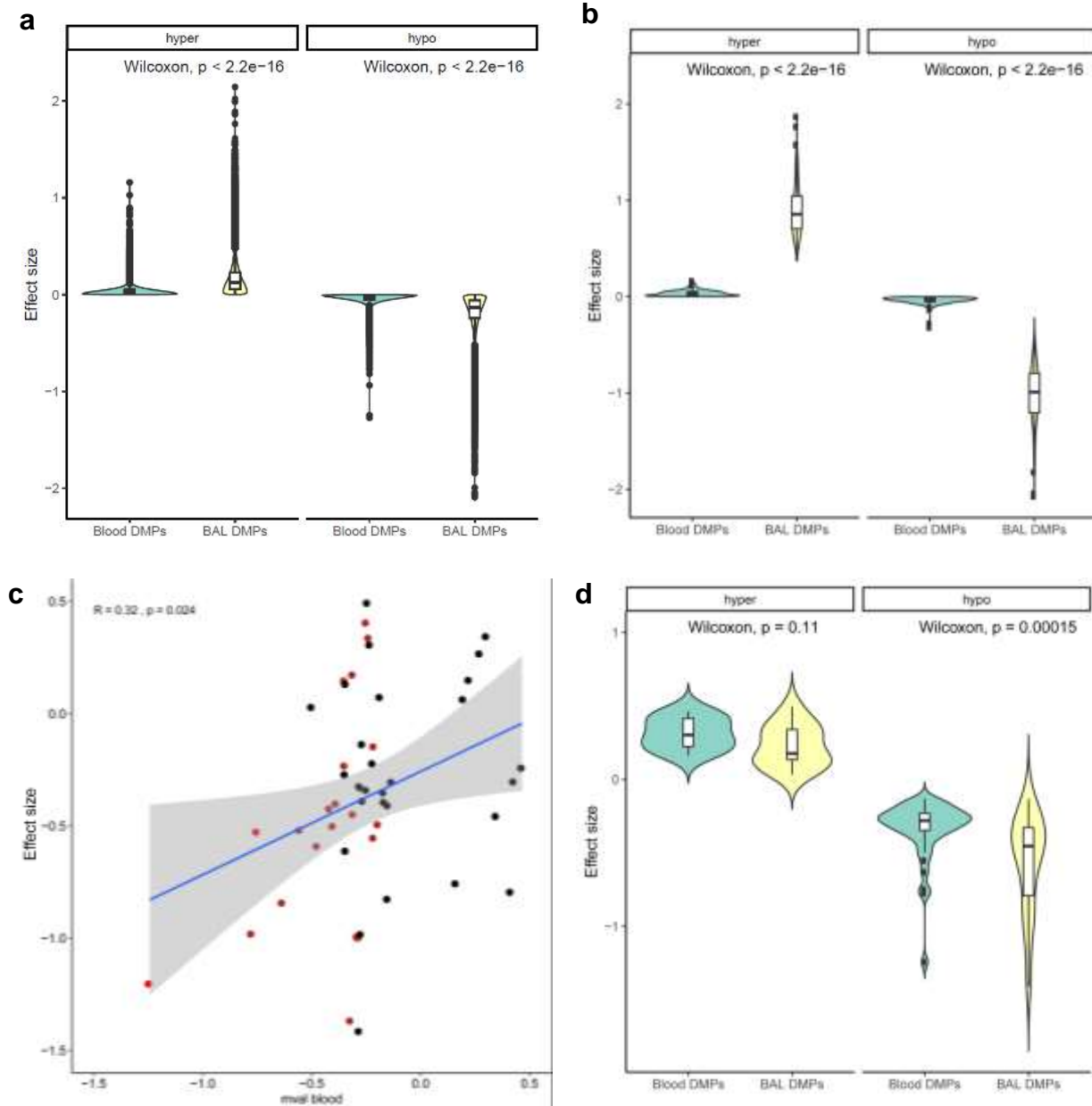
d



Supplementary Figure 2. Overlap of smoking-associated changes in Multiple Sclerosis (MS) patients and healthy controls (HC).

a-c. Distribution (violin plot, left) and correlation (scattered plot, right) of effect size ($\Delta\beta$ -values) in MS and HC at: **(a)** smoking-associated BS-DMPs in MS patients (MS-S vs MS-NS, adjusted $P < 0.05$) but not significant in HC (HC-S vs. HC-NS, not significant), **(b)** smoking-associated BS-DMPs in HC (HC-S vs. HC-NS, adjusted $P < 0.05$) but not significant in MS (MS-S vs. MS-NS) and **(c)** smoking-associated BS-DMPs in both MS and HC individuals, with delta.beta and beta-values illustrated in the left and right panels, respectively. **d.** Venn diagram of the smoking-associated BS-DMPs and 5mC-DMPs (adjusted $p < 0.05$) in MS patients (MS-S vs-MS-NS) and HC (HC-S vs. HC-NS).

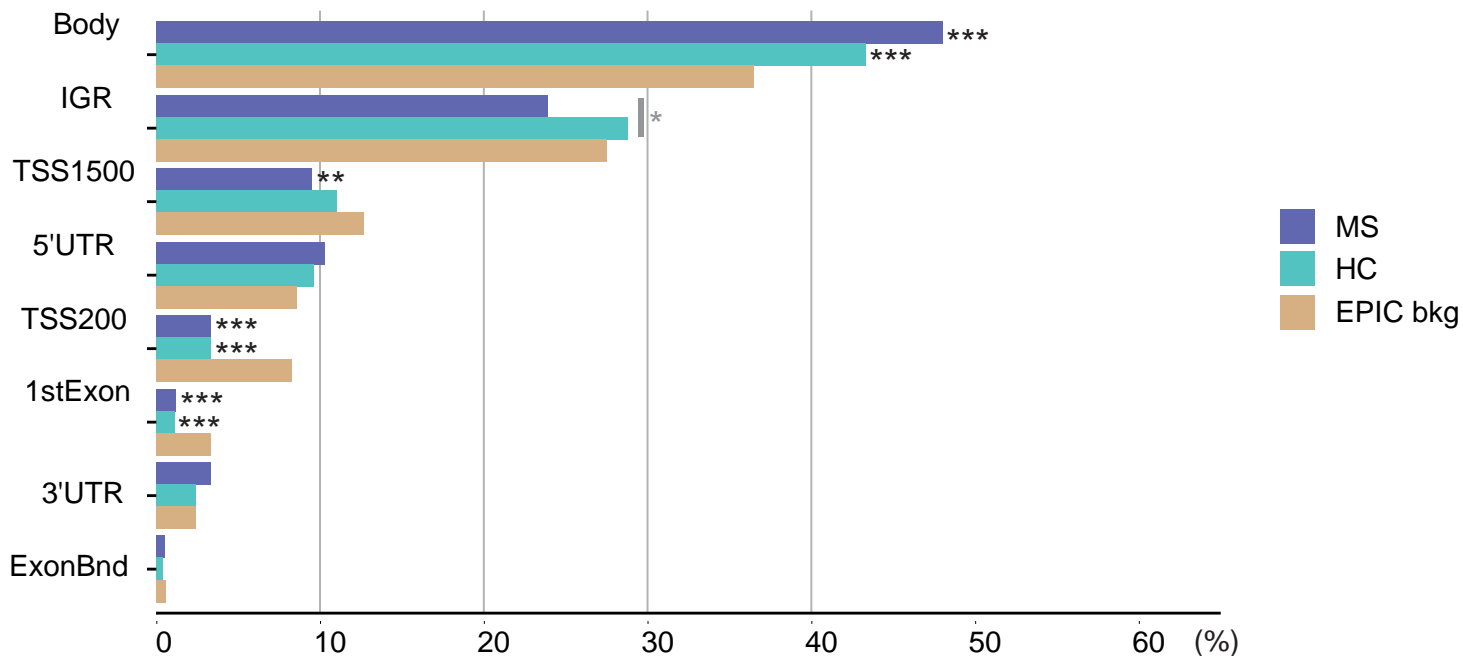
NEW Supplementary Figure 3



Supplementary Figure 3. Smoking effect in blood DMPs and BAL BS-DMPs of MS individuals.

a. Violin plot showing effect size at probes shared between BAL and blood ($n=382277$). **b** Violin plot representing all significant BS-DMPs in BAL with the corresponding probes in blood ($n=507$). **c.** Correlation scatterplot of blood BS-DMPs effect sizes (from M values) at $FDR < 0.05$ ($n = 50$) on the x-axis, and overlapping BAL BS-DMPs values on the y-axis. Red dots represent overlap with significant BAL BS-DMPs at $FDR < 0.05$, while dark red dots represent BAL BS-DMPs at $P < 0.05$. **d.** Violin plot showing effect size at significant BS-DMPs in blood with the corresponding probes in BAL ($n=50$). Effect size is based on M-values.

Supplementary Figure 4



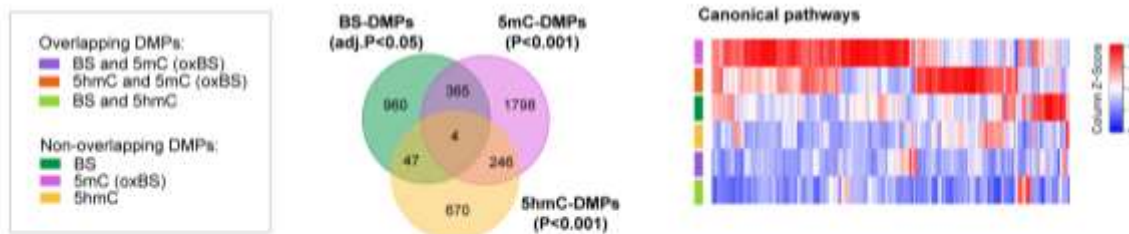
Supplementary Figure 4. Distribution of smoking-associated changes in Multiple Sclerosis (MS) patients and healthy controls (HC) across gene features.

Horizontal barplots representing relative frequencies of BS-DMPs associated with smoking in MS and HC across gene features (TSS1500, TSS200, 1stExon, 5'UTR, Body, 3'UTR, ExonBnd, IGR). Enrichment/depletion analysis was performed using Chi-square test on frequencies, with P-values adjusted for multiple testing (Bonferroni). Asterisk symbols in black (*) represent significance levels from Bonferroni-adjusted Chi-square tests of smoking-associated MS and HC BS-DMP profiles when compared to the EPIC array probe background. Asterisk symbols in grey * represent Bonferroni-adjusted significance levels from smoking-associated BS-DMPs of MS compared to those of HC.

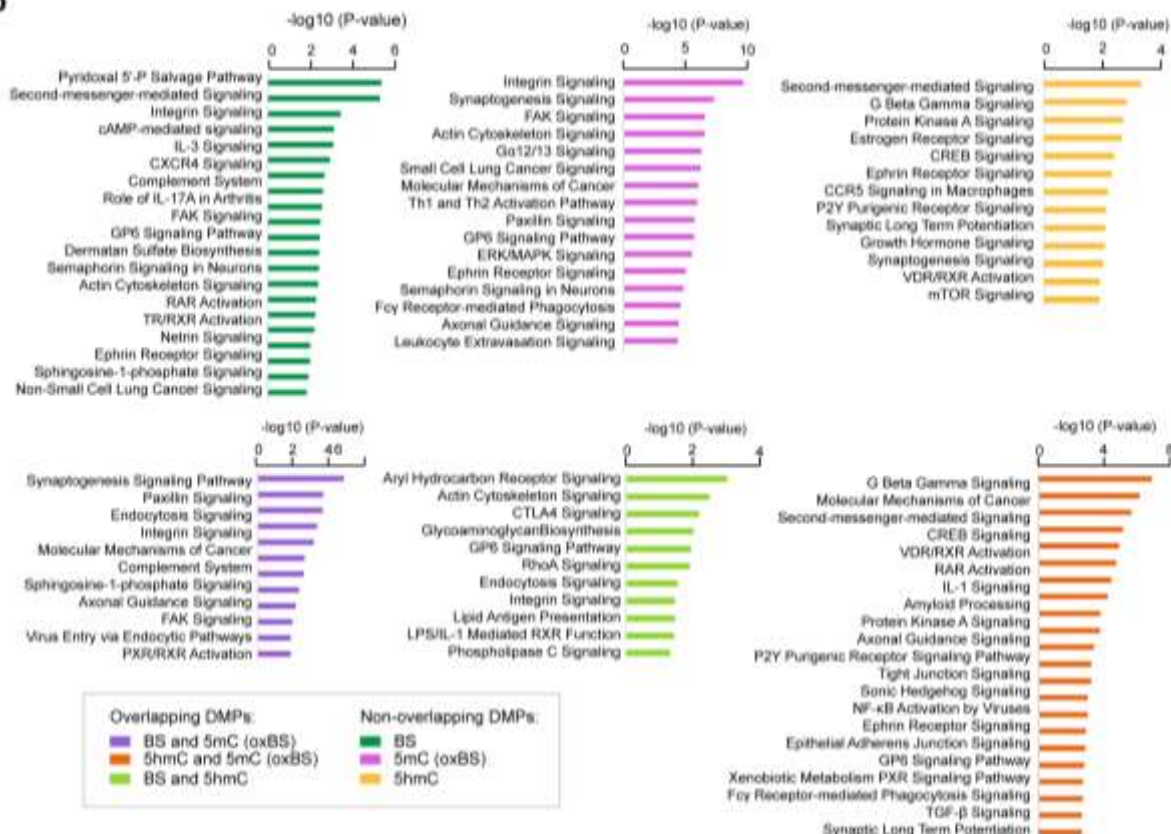
*P-value < 0.05, **P-value < 0.01, ***P-value < 0.001.

Supplementary Figure 5

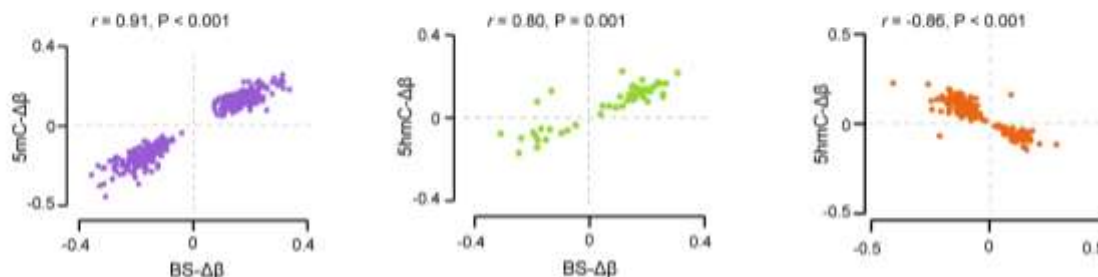
a



b



c



Supplementary Figure 5. Gene ontology (GO) analysis of overlapping and non-overlapping smoking-associated BS, 5mC and 5hmC changes in MS patients.

a. Venn-diagram illustrating the overlap of DMPs between BS (adjusted $P < 0.05$), oxBS (5mC) and 5mC or 5hmC ($P < 0.001$) and heatmap of overlapping Canonical pathways (from Ingenuity Pathway analysis, IPA) with blue to red colors low to high enrichment $-\log_{10}(P\text{-value})$. **b.** Top Canonical Pathways (IPA) enriched in overlapping/non-overlapping DMPs. **c.** Correlation of $\Delta\beta$ -values between overlapping BS-5mC (left panel), BS-5hmC (middle panel) as well as 5mC-5hmC (right panel) DMPs. P-values and Spearman's rank correlation coefficient (r) are given.